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S5750667: System to detect protein-A interactions

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Field of Search:

536/23.1 435/006

Abstract:

A method for detecting an interaction between an RNA-binding protein and a test RNA molecule is disclosed. This method comprises providing a host cell containing a detectable gene. The detectable gene expresses a detectable protein when the detectable gene is activated by an amino acid sequence including a transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the detectable gene. First, second and third chimeric genes are also provided. The first chimeric gene comprises a DNA-binding domain that recognizes a binding site on the detectable gene in the host cell and a first RNAbinding domain. The second chimeric gene comprises a transcriptional activation domain and a second RNA-binding domain. The third chimeric gene comprises a first RNA sequence capable of binding to either the first or second RNA-binding and a second RNA sequence to be tested for interaction with the RNA-binding protein not bound to the first RNA sequence. Interaction between both the first RNAbinding domain and the hybrid RNA and the second RNA-binding domain and the hybrid RNA in the host cell causes expression of the detectable gene.

Attorney, Agent, or Firm: Primary/Assistant Examiners:

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Related Applications:

| Application Number | ApplDate | Patent | Issued | Title |
|-----------------------|----------------|-----------|--------|---|
| US1995000409561 | 1995-03- 23 | US5610015 | 1 | System to detect protein-RNA interactions |

U.S. References:

(No patents reference this one)

| Patent | Issued | Inventor(s) | Title |
|-----------|---------|---------------|---|
| US5283173 | 2 /1994 | Fields et al. | System to detect protein-protein interactions |

Claim:

We claim:

- 1. A hybrid RNA molecule selected by detecting an interaction between an RNA-binding domain and a test RNA molecule, the method comprising:
 - (1) introducing into a host cell:
 - (a) a detectable gene wherein the detectable gene expresses a detectable protein when the detectable gene is activated by an amino acid sequence including a transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the detectable gene;
 - o (b) a first chimeric gene that encodes a first hybrid protein comprising:
 - (i) a DNA-binding domain that recognizes a binding site on the detectable gene in the host cell; and
 - (ii) a first RNA binding domain;
 - o (c) a second chimeric gene that encodes a second hybrid protein comprising:
 - (i) a transcriptional activation domain; and
 - (ii) a second RNA-binding domain;
 - o (d) a third chimeric gene that encodes said hybrid RNA comprising:
 - (i) a first RNA sequence that binds one of the first or second RNA-binding domains; and
 - (ii) a second RNA sequence to be tested for interaction with the RNA-binding domain not bound to the first RNA sequence;
 - wherein interaction between both the first RNA-binding domain and the hybrid RNA and the second RNA-binding domain and the hybrid RNA in the host cell causes the transcriptional activation domain to activate transcription of the detectable gene;
 - (2) subjecting the host cell to conditions under which the first hybrid protein, the second hybrid protein, and said hybrid RNA are expressed in sufficient quantity for the detectable gene to be activated;

- (3) determining whether the detectable gene has been expressed to a degree greater than expression of the detectable gene in the absence of interactions between both the first RNA-binding domain and said hybrid RNA and the second RNA-binding domain and said hybrid RNA; and
- (4) selecting said hybrid RNA.

This is a division of application Ser. No. 08/409,561 filed Mar. 23, 1995 U.S. Pat. No. <u>5,610,015</u>.

Foreign References:

none

(No patents reference this one)

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